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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/781,499	02/18/2004	Michel Chateau	CABR-029/US	6087

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EXAMINER	
SHAHNAN SHAH, KHATOL S	

ART UNIT	PAPER NUMBER
1645	

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07/07/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/781,499	CHATEAU ET AL.	
	Examiner	Art Unit	
	Khatol S. Shahnan-Shah	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 June 2009 and 18 June 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11,13 and 22-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9, 11, 13 and 22-32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Response to Amendment

1. Applicants' amendment after final of 06/03/2009 is acknowledged. The amendment has been entered into the record. Applicants' request for reconsideration of the finality of the rejection of the last Office action is persuasive and, therefore, the finality of that action is withdrawn.

2. Applicants' terminal disclaimer of 06/03/2009 is acknowledged. The terminal disclaimer has been approved by the office.

Status of claims

3. Claims 1-9, 11, 13 and 22-32 are pending and under consideration.

Rejection(s) Withdrawn

4. Rejection of claims 1, 2, 3, 8, 9, 11, 13, 14 and 22-32 provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 13-14 and 38-49 of copending Application No. 10/546,139, made in paragraph 7 of the office action mailed 2/03/2009 is withdrawn in view of applicants' terminal disclaimer of 06/03/2009.

5. Rejection of claims 1-4 and 8-9, 11, 13 and 14 under 35 U.S.C. 102 (b), made in paragraph 12 of office action mailed 1/18/2007 is withdrawn in view of applicants' arguments of 06/03/2009.

6. Rejection of claims 1-7 under 35 U.S.C. 102 (b), made in paragraph 13 of office action mailed 1/18/2007 is withdrawn in view of applicants' arguments of 06/03/2009.

7. Rejection of claims 22-32 under 35 U.S.C. 102 (b), made in paragraph 8 of office action mailed 2/03/2009 is withdrawn in view of applicants' arguments of 06/03/2009.

New Rejection(s)

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Claims 1-4, 8, 9, 11, 13 and 22-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Richaud et al. (J. Biological Chemistry. December 25, 1993; 268(36):26827-26835) in view of Short et al.

(US2005/0124010, published June 19, 2005 and priority date of September 2000).

The claims are drawn to a method for producing an evolved microorganism, comprising:

a) generating a directed genetic modification in a gene of interest in an initial microorganism to yield a modified microorganism wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, impairing the ability of the modified microorganism to grow;

b) culturing the modified microorganism obtained in step (a) on said defined medium, allowing the modified microorganism to evolve a compensatory metabolic pathway to compensate for the impaired growth, wherein the defined medium can contain a co-substrate promoting the evolution; and

c) selecting an evolved microorganism from step (b) able to grow on said defined medium; wherein a compensatory metabolic pathway is evolved allowing the evolved microorganism to proliferate on the defined medium.

Richaud et al. teach "disrupting the metC gene" (abstract) of *E. coli,* which the examiner interprets as satisfying the limitations directed to "generating a directed genetic modification in a gene of interest in an initial microorganism," as described in part a) of claim 1. Richaud et al. teach "a latent metabolite could under certain circumstances fulfill an essential need in cell chemistry, the way would be open for establishing a biosynthetic pathway *de novo"* (page 26827, co1.1), which satisfies the limitations of part b) claim 1, directed to evolution of a compensatory metabolic pathway. Richaud et al. also teach "expansion of thioether biosynthesis in *Escherichia coli* generates sulfur-containing amino acids that can replace meso-diaminopimelate, the essential amino acid used for cross-linking the cell wall," and "as a result, meso- lanthionine and L-allo-cystathione were produced endogenously and incorporated in the peptidoglycan, thereby enabling *E.coli* strains auxotrophic for diaminopimelate to grow in its absence" (abstract), which the examiner interprets as satisfying the limitations of part c) of claim 1 directed to "wherein at least one protein has evolved in the metabolic pathway compensating for the inhibition allowing the modified microorganism to proliferate." Richaud et al. describe this process, "techniques of metabolic engineering can be applied to evolving the chemical constitution of living cells beyond its present state" (abstract), which is similar to the broad outline of the instant invention provided by the specification. Furthermore, Richaud et al. teach "a metC mutation enhances the growth of *dap* strains exogenously supplied with L-lanthionine, meso-lanthionine, or L- allo-cystathionine as the cross-linking amino acid' and is absolutely required for growing such strains with exogenous L-cystathionine (a precursor of **methionine**) see page 26827 and (Table VI). The broad activity of cystathionase, which is indeed known to degrade generically L-cysteine thioethers in vitro, can thus be rationalized as fulfilling a corrective task, which adds to the biosynthetic function of the enzyme in *E. coli* metabolism " (page 26834, col.1, parag.1), which the examiner interprets as satisfying the limitations of part a) and b) of claim 1, directed to "wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, wherein the ability of the

modified microorganisms to grow is impaired" and "wherein the defined medium can contain a co-substrate." Richaud et al. further indicate, these strains can thus be viewed as having undergone an evolutionary commitment to use cystein thioethers for building their cell wall. Although this commitment did not result from natural selection but was rationally set up in their genome, the fitness of the committed strains might now be improved my natural selection" (page 26834, col.2, parag.1).

Claims 22 and 25 are directed to the method of claim 1, wherein the gene of interest (claim 22) or evolved protein (claim 25) is homologous or heterologous. The specification teaches "this invention also concerns a method comprising an additional step al) in which at least one heterologous gene coding for a heterologous protein is introduced, which heterologous gene is intended to cause the evolution of a new metabolic pathway" (page 2, lines 9-11). Richaud et al. teach "jointly overexpressing the *metB* gene coding for L-cystathionine γ-synthase and disrupting the *metC* gene" abstract). In this case, the disrupted *metC* gene is the homologous gene of interest and the overexpressed *metB* gene the heterologous evolved protein.

Claim 23 is directed to the method of claim 1, wherein the defined medium is substantially free of the substrate the production or consumption of which is inhibited in the modified microorganism. In the example of Richaud et al., the substrate is meso-diaminopimelate. Richaud et al. teach "thereby enabling *E.coli* strains auxotrophic for diaminopimelate to grow in its absence" (abstract). It seems that the substrate is not present in the medium of these *E.coli* strains.

Claim 24 is directed to the method of claim 1, wherein in step (b) a co-substrate ~s added to the defined medium. Richaud et al. teach "growth of *E. coli* mutants bearing a deletion of the diaminopimelate pathway in the presence of lysine and in the absence of diaminopimelate there provide an inescapable selection screen for the endogenous production of diaminopimelate substitutes."

(bottom page 26827 bridging 26828). This seems to satisfy the limitations of claim 24.

Claim 26 is directed to the method of claim 1, wherein the genetic modification comprises the directed mutation or deletion of a gene of interest or the directed modification of a promoter in the gene of interest. Richaud et al. teach "disrupting the metC gene" (abstract) of *E. coli,* which the examiner interprets as satisfying the limitations directed to "generating a directed genetic modification in a gene of interest in an initial microorganism," as described in part a) of claim 1.

Claim 27 is directed to the method of claim 1, wherein the genetic modification consists in the removal of most of the gene of interest. Richaud et al. teach "disrupting the metC gene" (abstract) of *E. coli,* which the examiner interprets as satisfying the limitations directed to "generating a directed genetic modification in a gene of interest in an initial microorganism," as described in part a) of claim 1. The type of mutation does not seem to be particularly important to the practice of the method. Any type of null mutant, whether created by a deletion, point mutation, etc would be obvious in light of the teachings of Richaud et al.

Claim 28 is directed to the method of claim 1, wherein the gene of interest is replaced with a selection marker gene. The type of mutation does not seem to be particularly important to the practice of the method. Any type of null mutant, whether created by a knockout by replacing the gene of interest with a selection marker, or by any other known means, would be obvious in light of the teachings of Richaud et al. *metC* gene" (abstract). In this case, the overexpressed *metB* gene encodes the heterologous evolved protein.

Claim 29 is directed to the method of claim 1, wherein the microorganism is selected among bacteria, yeasts, and fungi. Richaud et al. teach a method which uses *E. coli.*

Claim 30 is directed to the method of claim 1, wherein the microorganism is...[various microorganisms] including *Escherichia sp.* Richaud et al. teach a method which uses *Escherichia coli.*

Claim 31 is directed to the method of claim 1, wherein the microorganism is E. *coli* and *C. glutamicum.* The instant specification does not describe a method that uses two different microorganisms, so the examiner is interpreting the instant claim as reciting "or" rather than "and." In particular, the specification describes using either E. *coli* or *C. glutamicum* on page 8, lines 8-10 of the specification. Richaud et al. teach a method which uses *E. coli.*

Richaud et al. does not teach all the limitations of claims 9 and 13 . The only element of claims not taught by Richaud et al. is part d), directed to isolation of the evolved gene or protein.

However, Short et al. teach "directed evolution...generating transgenic organism, such as microbe" (abstract). Short et al. further teach isolating cells which produce a desired metabolite (paragraphs 1062-1063) and also teach measuring metabolic parameters such as growth, as well as "changes in the expression of the polypeptide can be measured by any method, e.g., a one-dimensional gel electrophoresis,...western blot" (parag.1070). Short et al. teach that cystathionine synthase is an example of the gene or gene product used in their methods.

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to combine the teachings of Richaud et al and Short et al. so that the evolved protein produced by the microorganisms of Richaud et al are isolated. The person of ordinary skill in the art would have been motivated to make those modifications because Short et al. suggest measuring expression levels of the evolved protein, as an alternative or in addition to measurement such as growth on defined media. The skilled artisan would have had a reasonable expectation of success in combining the teachings of Richaud et al. and Short et al. because each

of these teachings generated evolved microorganisms and discuss the proteins which make possible the growth of the auxotrophic organisms. Therefore the method as taught by Richaud et al. in view of Short et al. would have been *prima facie* obvious over the method of the instant application.

10. Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Richaud et al. (J. Biological Chemistry. December 25, 1993; 268(36):26827-26835) in view of WO 93/177112 published 2 September 1993.

The claims are drawn to a method for producing an evolved microorganism, comprising:
a) generating a directed genetic modification in a gene of interest in an initial microorganism to yield a modified microorganism wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, impairing the ability of the modified microorganism to grow;
b) culturing the modified microorganism obtained in step (a) on said defined medium, allowing the modified microorganism to evolve a compensatory metabolic pathway to compensate for the impaired growth, wherein the defined medium can contain a co-substrate promoting the evolution; and
c) selecting an evolved microorganism from step (b) able to grow on said defined medium; wherein a compensatory metabolic pathway is evolved allowing the evolved microorganism to proliferate on the defined medium.

Richaud et al. teach "disrupting the metC gene" (abstract) of *E. coli,* which the examiner interprets as satisfying the limitations directed to "generating a directed genetic modification in a gene of interest in an initial microorganism," as described in part a) of claim 1. Richaud et al. teach "a latent metabolite could under certain circumstances fulfill an essential need in cell chemistry, the way would be open for establishing a biosynthetic pathway *de novo"* (page 26827, col.1), which satisfies the limitations of part b) claim 1, directed to evolution of a compensatory metabolic pathway. Richaud et al. also teach "expansion of thioether biosynthesis in *Escherichia coli* generates sulfur-containing amino acids that can replace meso-

diaminopimelate, the essential amino acid used for cross-linking the cell wall," and "as a result, meso- lanthionine and L-allo-cystathione were produced endogenously and incorporated in the peptidoglycan, thereby enabling *E.coli* strains auxotrophic for diaminopimelate to grow in its absence" (abstract), which the examiner interprets as satisfying the limitations of part c) of claim 1 directed to "wherein at least one protein has evolved in the metabolic pathway compensating for the inhibition allowing the modified microorganism to proliferate." Richaud et al. describe this process, "techniques of metabolic engineering can be applied to evolving the chemical constitution of living cells beyond its present state" (abstract), which is similar to the broad outline of the instant invention provided by the specification. Furthermore, Richaud et al. teach "a metC mutation enhances the growth of *dap* strains exogenously supplied with L-lanthionine, meso-lanthionine, or L- allo-cystathionine as the cross-linking amino acid' and is absolutely required for growing such strains with exogenous L-cystathionine (a precursor of **methionine**) see page 26827 and (Table VI). The broad activity of cystathionase, which is indeed known to degrade generically L-cysteine thioethers in vitro, can thus be rationalized as fulfilling a corrective task, which adds to the biosynthetic function of the enzyme in *E. coli* metabolism " (page 26834, co1.1, parag.1), which the examiner interprets as satisfying the limitations of part a) and b) of claim 1, directed to "wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, wherein the ability of the modified microorganisms to grow is impaired" and "wherein the defined medium can contain a co-substrate." Richaud et al. further indicate, these strains can thus be viewed as having undergone an evolutionary commitment to use cystein thioethers for building their cell wall. Although this commitment did not result from natural selection but was rationally set up in their genome, the fitness of the committed strains might now be improved my natural selection" (page 26834, col.2, parag.1).

Richaud et al. does not teach all the limitations of claims 5-7 .

However, WO 93/177112 teaches a preparation of evolved microorganisms permitting a modification of metabolic pathways i.e. biosynthesis pathway of amino acids (see abstract and amended claims). WO 93/177112 teaches preparing a modified microorganism by genetic modification of cells of an initial microorganism so as to inhibit the production or consumption of a metabolite (methionine) when that microorganism is grown on a defined medium (see claims). WO 93/177112 teaches preparing a modified microorganism by genetic modification of cells of an initial microorganism see amended claim 1. WO 93/177112 teaches culturing the modified microorganism thereby obtained on said defined medium to cause it to evolve, where the defined medium can contain a co-substrate to allow such evolution and c) selecting a modified microorganism able to grow on said defined medium, if necessary with a co-substrate, see claims specially claim 1. **WO 93/177112 teaches limitations of claims 5-7 wherein the metabolic pathway consumes NADPH (see figure 1)**. WO 93/177112 teaches biosynthesis pathway of amino acids, methionine (see title and abstract).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to combine the teachings of Richaud et al. and WO 93/177112. so that the modified metabolic pathway consume NADPH. The person of ordinary skill in the art would have been motivated to make those modifications because Richaud et al. that metabolites have been generated by recombining biosynthetic pathways see Richaud et al. page 26827 . The skilled artisan would have had a reasonable expectation of success in combining the teachings of Richaud et al. and WO 93/177112.. because each of these teachings generated evolved microorganisms. Therefore the method as taught by Richaud et al. in view of WO 93/177112. would have been *prima facie* obvious over the method of the instant application.

Conclusion

11. No claims are allowed.

12. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Khatol Shahnan-Shah whose telephone number is 571-272-0863. The examiner can normally be reached on Monday-Friday 7:30 AM-5:00 PM If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert B. Mondesi can be reached on 571-272-0956.

The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

/Khatol S Shahnan-Shah/
Examiner, Art Unit 1645
June 30, 2009
/Robert B Mondesi/
Supervisory Patent Examiner, Art Unit 1645